Trace Components of the Flavor Fraction of Maple Syrup

SUMMARY—An exhaustive chloroform extraction of maple syrup removed the maple flavorants. The extract was analyzed in part by a gas chromatograph-mass spectrometer tandem procedure. Several previously undetected flavor-related compounds were found in trace amounts. Among these were the aromatic compounds acetovanillone, guaiacyl acetone and vanilloyl methyl ketone. These aromatics could have resulted from the ethanolysis of ligneous material previously reported in maple sap. Sugar degradation products found were furfural, hydroxymethylfurfural, lactic acid and levulinic acid. These indicate that the products of caramelization also are part of the maple flavorants.

Acids found, in addition to those above, were the C_5 to C_9 aliphatic acids and oxalic, fumaric and malic acids. All of the acid occurred as ethyl esters resulting from unintentional esterification during extraction. The C_5 to C_9 acids may be artifacts perhaps derived from the vegetable oil used as antifoaming agent in syrup processing.

INTRODUCTION

EARLIER PUBLICATIONS have described the use of chloroform to isolate the maple flavor from the sugar in maple syrup (Underwood *et al.*, 1961, 1963). Vanillin, syringaldehyde and dihydroconiferyl alcohol were separated and identified from chloroform extracts, using both column and gas chromatography. Later, through the use of more refined techniques, the more volatile ketolic compounds, methylcyclopentenolone, acetoin and acetol were identified in such an extract (Filipic *et al.*, 1965).

However, many small peaks on the gas chromatograms of the chloroform extracts made in these studies indicated the presence of more flavor components in trace amounts. To identify as many of these compounds as possible analysis has been made of a chloroform extract of maple syrup. The concentrated extract and some individual gas chromatographic fractions were subjected to a GLC-mass spectrometer tandem procedure which proved most successful with the more volatile components of the extract. This paper reports the results of the work covering isolation and identification of 34 different compounds, a few of which are only tentatively identified.

EXPERIMENTAL

Preparation of the chloroform extract

To insure obtaining the maximum amount of the chloroform soluble materials from the maple syrup, a more rigorous extraction procedure was used in this study than in earlier work. A No. 1 or A grade commercial maple syrup containing a high level of distinctive maple flavor was used for this study.

Forty gal of syrup were extracted in approximately 3 gal batches with analytical grade chloroform using an all-glass, steam-heated, 50 L liquid-liquid extraction apparatus. Each 3 gal batch was extracted continuously for 24 hr with fresh solvent from the 25 L reservoir of $CHCl_3$ in the extraction. Thus, the syrup was subjected to a longer period of treatment at a higher temperature (25–60°C) than earlier extractions. Further, the $CHCl_3$ solubles removed from the syrup were subjected to the temperature of boiling $CHCl_3$ (60°C) in the solvent reservoir.

The solvent in the reservoir was used to treat three batches of syrup before it was removed for a fresh charge. The 25 L of solvent thus obtained was concentrated to about 4 L in the solvent reservoir of the apparatus by distilling off $CHCl_3$ using steam heat. The four 4 L volumes of extract resulting from the treatment of the 40 gal of syrup were combined and allowed to evaporate at room temperature in a hood through which a stream of air was moving. The extract was thus concentrated to 2 L at which point the solution became saturated in respect to the least soluble of the constituents in it. This concentrate was stored in a glass-stoppered bottle.

Ether-treated chloroform extract

In earlier work with $CHCl_3$ extracts of maple syrup, it was learned that much improved GLC separations could be made if the lignin-like material in $CHCl_3$ were removed. Therefore, a 500 ml aliquot of the gross $CHCl_3$ concentrate was shaken with 2000 ml of peroxide-free diethyl ether and the precipitate filtered off using a sintered glass filter. The filtered solution was then concentrated to 100 ml as described for the original $CHCl_3$ extract. This purified extract was again stored in a glass-stoppered bottle for the GLC work.

Gas chromatographic fractionation

Thirty-five gas chromatographic fractions were collected by a previously described technique (Filipic *et al.*, 1965). A dual column chromatograph with the F & M Model 720 thermal conductivity detector was used. The dual columns were packed with 20% Carbowax 20M on 60–80 mesh acid-washed Chromosorb W and the oven programmed from 50°C to 240°C at 4° per min. The initial temperature was held for 16 min after injection to permit complete elution of the solvent and the final temperature for 50 min to allow for elution of the major high boilers. The reproducible chromatogram obtained is illustrated in Fig.1. Fig. 2, 3 and 4 show the temperature range of each fraction in greater detail.

Fractions 1–9 were rechromatographed on the same Carbowax 20M column; fractions 10–34 on a silicone SE-30 column. As in the previous study, subfractions were collected in melting point capillary tubes. In all cases

where sufficient isolate was collected, the infrared and mass spectra were obtained. In addition, fractions 1–14 were analyzed by the combined gas chromatogram-mass spectrometer technique.

This gas chromatograph was an F & M 810 equipped with a flame ionization detector and a 1:10 splitter at the column exit. A portion of the effluent from the chromatograph was diverted into the ion source of a CEC-103 C mass spectrometer via a heated valving system. No helium separator was used and that amount of effluent sufficient to raise the pressure to 7×10^{-6} torr was introduced.

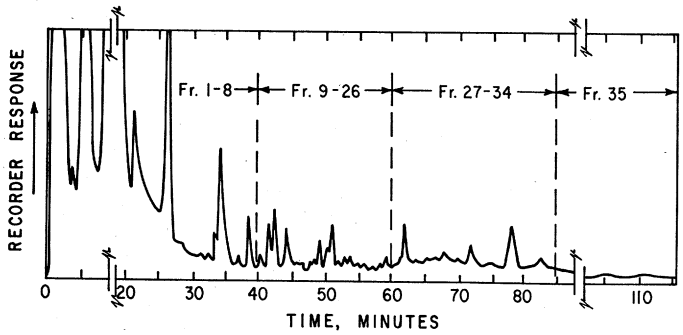


Fig. 1. The chromatogram of the exhaustive extract of maple syrup (attenuation 4x).

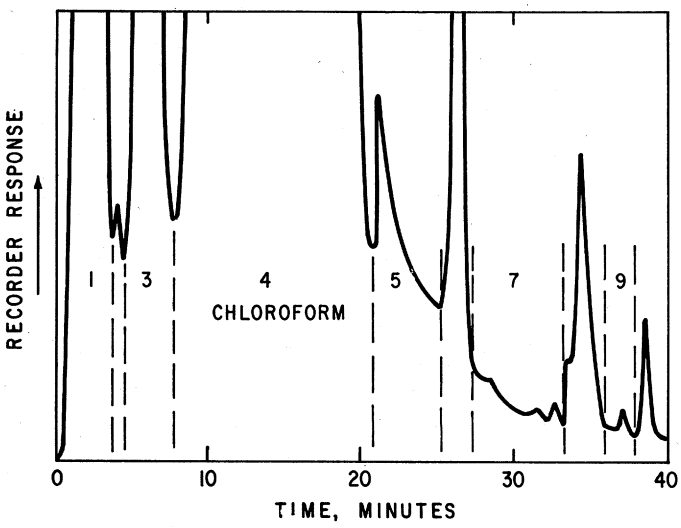


Fig. 2. Fractions 1–10, illustration of retention range of collection.

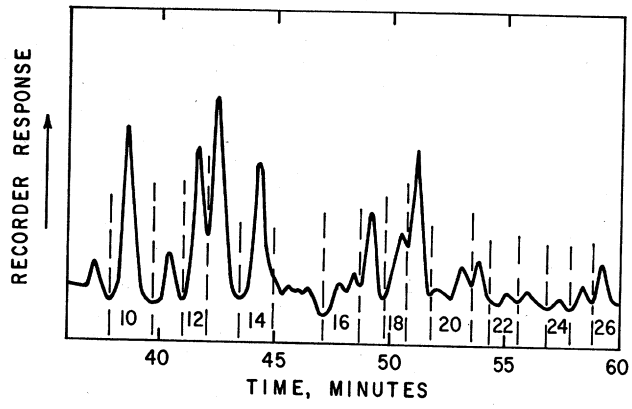


Fig. 3. Fractions 9–26, illustration of retention range of collection.

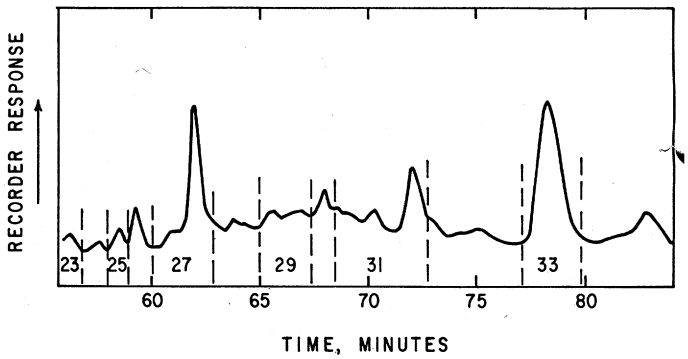


Fig. 4. Fractions 23–34, illustration of retention range of collection.

RESULTS AND DISCUSSION

THE COMPOSITION of all the gas chromatographic fractions is summarized in Table 1. Whenever possible the spectral methods of identification were supplemented either by cochromatography of a standard with the unknown or by a comparison of retention times.

Spectral interpretations

The majority of the compounds listed in Table 1 are the products of ethanolysis resulting from the prolonged extraction by refluxing chloroform containing 1% ethanol as a preservative. In spite of this solvent interaction most of the compounds identified can be classified into four major categories: solvent-related impurities, contaminants and artifacts, constituents of ligneous origin and constituents of carbohydrate origin.

Three of the components cannot be so described; these were the ethyl esters of nonvolatile acids present in maple syrup (Porter *et al.*, 1951) diethyl malate, diethyl fumarate and ethoxydiethylsuccinate. Some of the identifications are tentative because standards were not available for comparison of spectral and chromatographic data.

A component of fraction 26 is suspected to be ethoxymethylfurfural (EMF) mainly on the basis of infrared and mass spectral comparisons with hydroxymethylfurfural (HMF). The infrared spectrum of EMF differed notably from that of HMF by the absence of a hydroxyl band near 3.0 μ and the appearance of a strong band at 9.1 μ, in the C-O-C asymmetric stretching region of aliphatic ethers (Colthup *et al.*, 1964). However, the normally strong hydrogen stretching vibrations of the furan ring in the 12–13 μ region were of low to medium intensity, possibly due to impurity of the isolate. The mass spectrum showed a parent peak of 154 with some of the prominent fragments having masses of 125 (M-29), 109 (M-45), 97 (M-57) and 39 which would be expected from this type of substituted furan (Grigg *et al.*, 1965) as illustrated in Fig. 5.

The major component of fraction 30 was suspected to be ethoxy diethyl succinate, which could result from the further ethanolysis of diethyl malate. Some corroboration of this assumption can be elicited from a comparison of the

Table 1. Composition of GLC fractions and bases of identification.

Fraction number	Constituents	Spectral methods		GLC Methods	
		Infrared	Mass spec.	20 M	SE 30
1	Ether*	....	G	C	....
2	Ethyl acetate*	....	....	C	C
3	Ethyl alcohol*	S	G, M	R	....
4	Chloroform*	....	G	R	....
5	Two silicones*	....	G	....	....
6	Diethyl carbonate*	S	G, M	C	....
7	Ethyl valerate	....	G	R	....
	Several silicones*	....	G	....	....
8	A chlorinated artifact*	....	G	....	....
	A silicone	....	G	....	....
	Ethyl caproate	....	G	R	....
9	Acetol*	....	G	C	C
10	Ethyl lactate*	S	G	R	....
	Ethyl heptoate	....	G	R	....
11	A chlorinated artifact*	....	G	....	....
12	Phenetole	....	G	R	....
	A silicone*	....	G	....	....
13	Ethyl octanoate	....	G	R	....
	A chlorinated artifact*	....	G	....	....
14	Diethyl oxalate*	....	G	R	....
	Furfural	....	G	R	....
	1,1,2,2-tetrachloroethane	....	G	R	....
15	Ethyl nonanoate*	....	M	R	....
	A silicone	K	....	....	....
16	Two silicones*	K	M	....	....
17	Ethyl levulinate*	....	M	R	....
18	A hydrocarbon*	K	M	....	....
19	Diethyl fumarate*	K	M	R	....
20	Unknown* (1)	....	M	....	....
21	A hydrocarbon*	K	M	....	....
22	Unknown* (2)	....	M	....	....
23	Methylcyclopentenolone*	....	M	C	R
24	No major component	....	....	....	....
25	Unidentified*	....	....	....	....
26	Ethoxymethylfurfural (5)	K	M	....	....
27	Diethyl malate*	K	M	C	R
28	No major component	....	....	....	....
29	No major component	....	....	....	....
30	Ethoxydiethyl succinate* (5)	K	M	....	....
	Ethyl palmitate	K	M	R	....
31	Para ethoxy phenol*	K	M	R	....
	Ethyl phthalate	K	M	R	....
32	Hydroxymethylfurfural	K	M	C	....
	Ethyl stearate	K	M	R	R
	Ethyl oleate	K	M	R	R
33	Vanillin*	K	M	C	R
34	Acetovanillone	K	M	C	R
	Guaiacyl acetone	K	M	....	....
	Ethyl vanillate	K	M	C	R
	Unknown (3)	....	M	....	....
	Chloropropyl guaiacol (5)	K	M	....	....
35	Vanilloyl acetyl	K	M	....	....
	Syringaldehyde*	K	M	C	R
	Dihydroconiferyl alcohol*	K	M	C	R
	Unknown (4)	....	M	....	....
	Butylphthalylbutylglycollate	K	M	....	....

* Major constituent in fraction.
S Sealed cell.
K Micro KBr pellet.
G Tandem GC-MS.
M Mass spectrograph of a collected GLC fraction.
C Standard cochromatographed with sample.
R Retention time of sample and standard agree.
(1) MS indicates molecular weight of 176.
(2) MS indicates molecular weight of 170.
(3) MS indicates molecular weight of 224.
(4) MS indicates molecular weight of 210.
(5) Identification is tentative because no standard was available for comparison of spectral and chromatographic data.

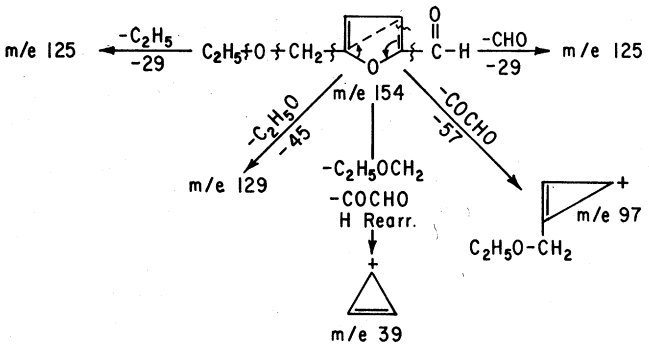
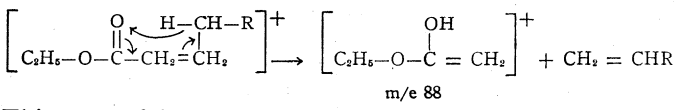


Fig. 5. Probable mass spectral fragmentations of ethoxymethylfurfural.

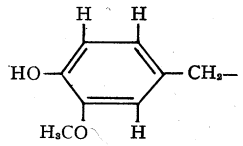
spectra in Fig. 6. Again the hydroxyl band is greatly diminished in the unknown. Also the band at 12.75 μ which could be ascribed to the CH_2 rocking frequency of an ethyl group (Colthup *et al.*, 1964) is significantly more intense in the unknown.

The mass spectrum is identical to that of diethyl malate with two slight exceptions, an increase in a small m/e 81 (the significance of which we have not deduced) and the disappearance of a small mass at 88. The m/e 88 is commonly found in the spectra of ethyl esters of fatty acids and can occur when a gamma hydrogen is available (Budzikiewicz *et al.*, 1964) as shown:



This type of fragmentation can occur with diethyl malate due to the availability of the hydroxyl hydrogen, but cannot occur and is absent in the spectra of diethyl fumarate and maleate. The parent peaks in the mass spectra of all four of these compounds are virtually absent and cannot be used to aid in structural identification.

The evidence for the tentative identification of chloropropylguaiacol in fraction 34 is based mainly on its mass spectrum. A strong parent peak at 200 m/e with a p + 2 peak of about one-third the intensity indicated a monochloro compound. The fragmentation at m/e 137 and below was very similar to compounds having the structure:



The > C-CH_2-CH_2-Cl group results in two bands (related to the presence of Cl) due to rotational isomers. The planar C-C-C-Cl trans zig-zag form absorbs near 726 cm^{-1} and the gauche C-C-C-Cl form near 649 cm^{-1} (Colthup *et al.*, 1964). Bands at these positions were detected in the infrared spectrum of this isolate. However, these bands were weaker than expected, possibly due to impurity of the isolate.

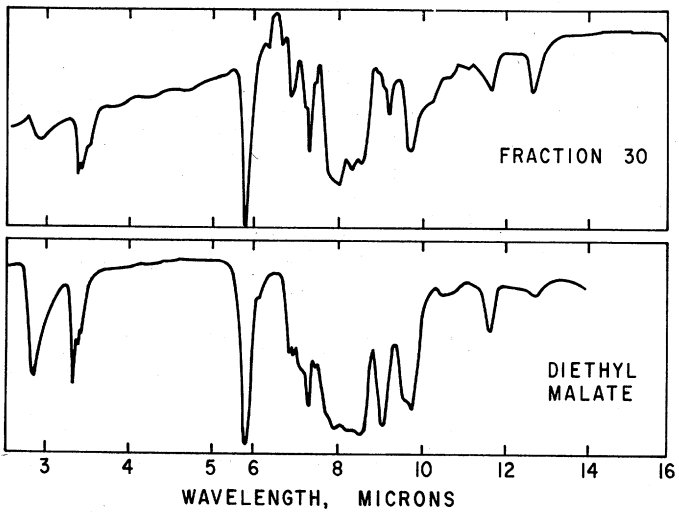


Fig. 6. A comparison of the infrared spectra of fraction 30 and diethyl malate.

Related experimental observations

Another observation may tend to support the foregoing conclusions. In prior extracts, the peak due to dihydroconiferyl alcohol (DHCA) was always more intense in the first chromatogram of the day. But in this extract, a peak in fraction 34 showed that effect and there was no increase in the DHCA peak. Apparently some nonvolatiles in the extracts slowly decompose in the injection port (kept at 140–160°C) to yield DHCA. In this extract, in which a large number of chlorinated artifacts were detected, a chlorinated decomposition product rather than DHCA, was the result.

Mass spectral analysis indicated that trace amounts of alpha ketols were apparently converted to diketones by the gas chromatographic fractionation. The p-2 peaks were far more intense at low concentrations than the expected parent peaks. This was true whether the compound was introduced to the ion source via the tandem GLC system or by the normal sample introduction system. This effect was noted with acetoin, acetol and 5-hydroxymethylfurfuryl, which is an alpha ketol via conjugation.

The final concentrate was also observed to be far less stable than prior extracts. In Fig. 7, the chromatogram of fraction 9 through fraction 23 of the extract, fractionated in this study, is compared to the same chloroform extract immediately after treatment with a fresh batch of ether. In the fresh batch, fractions 9 and 23, which contain acetoin, acetol and methylcyclopentenolone, are much larger. Fractions 17 and 19 which contain ethyl esters of levulinic and fumaric acids are much smaller.

Solvent-related impurities

Four of the compounds listed in Table 1 are related to the use of ether and chloroform solvents. The chloroform used for extraction contained 1% ethanol as a preservative. Whenever a heated maple syrup was extracted with this solvent, a relatively large amount of diethyl carbonate formed. Apparently, the solvent also contained a trace of 1,1,2,2 tetrachloroethane impurity. The mass spectra indicated the presence of several chlorinated artifacts, one of which is suspected to be chloropropyl guaiacol.

Contaminants and artifacts

The maple syrup analyzed in this study was of commercial origin and was extracted in large scale equipment.

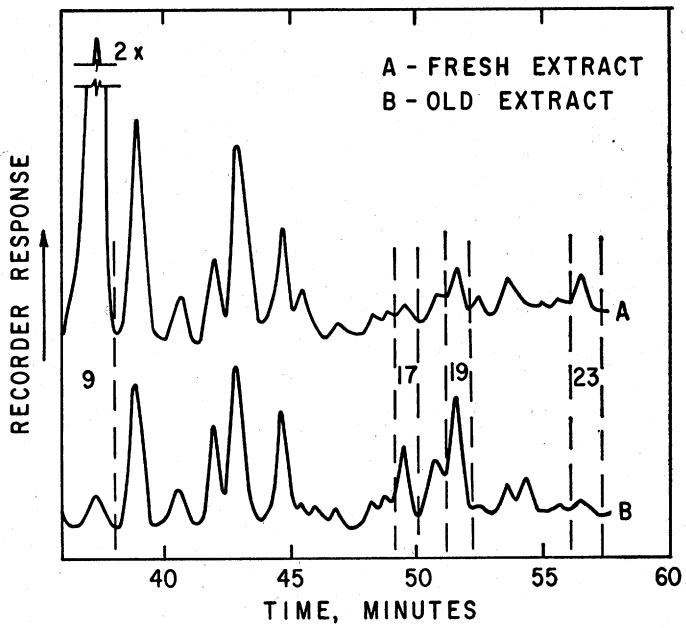


Fig. 7. Comparison of chromatograms obtained from fresh and old extracts of maple syrup.

For one or both of these reasons the extract contained a large number of trace contaminants consisting mainly of unidentified silicones and hydrocarbons. Phthalate esters were another type of contaminant; butylphthalyl butyl glycolate and diethyl phthalate (possibly not a contaminant, see below) are two that were identified in this extract.

Some of the ethanolysis products resulted from interaction with a contaminant. Corn oil was used as an antifoaming agent in the processing of the maple syrup extracted in this study. This accounts for the presence of ethyl oleate, palmitate and stearate. The ethyl esters of the C_5 to C_9 acids were also present, ethyl nonanoate in the greatest concentration. Such an effect points to the breakdown of unsaturated fatty acids, oleic and linoleic, present in the oil. The long chain fatty acid in a previous extract in which extensive ethanolysis did not occur probably was nonanoic acid (Filipic *et al.,* 1965). This compound was the major component in fraction 15 of that prior extract. Fig. 8 is a reproduction of the center portion of the chromatogram obtained with that extract. Phenol, a possible contaminant, was previously detected in unreacted form (fraction 14, Fig. 8) and this time in the form of two ethanolysis products, phenetole and paraethoxy phenol.

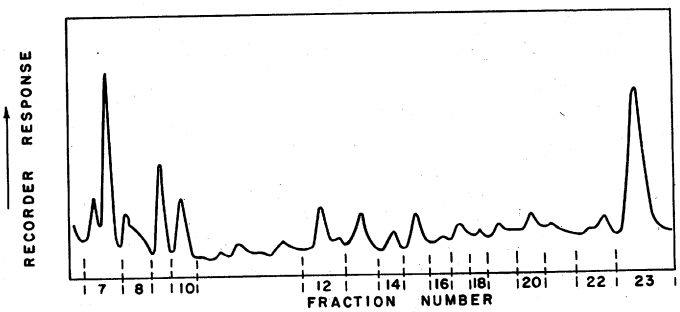


Fig. 8. Fractions 7–23, from a chromatogram of a different maple syrup extracted under milder conditions.

Constituents related to lignin

Vanillin, syringaldehyde and dihydroconiferyl alcohol have been previously reported and ascribed to degradation of ligneous material (Underwood *et al.,* 1964). Acetovanillone (in fraction 34) also is a lignin degradation product of frequent occurrence (Brauns *et al.,* 1960). Vanillin, ethyl vanillate, vanilloyl acetyl and guaiacyl acetone are all products to be expected from the ethanolysis of lignin (Fujii *et al.,* 1966). The unknown in fraction 34 (M.W., 224) may very well be another of Hibbert's ketones, one of the ethoxyguaiacylpropanones. Oxalic and phthalic acids, present here as ethyl esters, are among the chief oxidation products of lignin and wood (Pearl *et al.;* Brink *et al.,* 1966).

Constituents of carbohydrate origin

As might be expected, ethanolysis products of carbohydrate degradations were also present—ethyl lactate, ethyl levulinate and ethoxymethylfurfural. Other products of probable carbohydrate origin were acetol, furfuryl, methylcyclopentenolone and hydroxymethylfurfural.

In the chloroform extracts of any maple syrup that has been freshly extracted under milder conditions than here employed, the predominant constituent is acetol. Methylcyclopentenolone has always been a primary constituent. Inspection of the infrared data from our previous study indicates that the primary component in fraction 8 (Fig. 8) was furfural. Hydroxymethylfurfural has been found in this laboratory to be a major constituent of high flavored maple syrup (maple syrup given additional heat treatment).

Constituents of known flavor effect

In a recent review, Hodge (1967) describes the desirable degree of sugar caramelization to be that point at which fragrant notes are added to the sweetness of the sugar and before further heat treatments develop burnt, bitter and acrid flavors that overcome the sweet. In the same review a table describes the odor and taste of various carbohydrate caramelization and dehydration products. Of such products in the mild chloroform extraction of maple syrup, acetol has a sweet taste and other constituents such as acetoin, furfural and methylcyclopentenolone impart desirable characteristics. Compounds such as levulinic acid and hydroxymethylfurfural which impart sour and bitter flavor notes are present in low concentrations.

Of the compounds deriving from ligneous material in the maple sap, vanillin is apparently the most important with respect to flavor contribution. Because of the very wide range of odor and taste thresholds, one must be very careful in relating flavor effects to the concentration of the flavor components. However, in this case, methylcyclopentenolone (fraction 12, Fig. 8) and vanillin, (fraction 23, Fig. 8) two of the prominent constituents in the previous mild extraction, impart desirable notes to the flavor complex and are known to have extremely low flavor thresholds (Dow Chemical Company 1966).

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